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Commentary & View

Insights into intragenic and extragenic effectors of prion propagation using chimeric prion proteins

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The study of fungal prion proteins affords remarkable opportunities to elucidate both intragenic and extragenic effectors of prion propagation. The yeast prion protein Sup35 and the self-perpetuating *[PSI+]* prion state is one of the best characterized fungal prions. While there is little sequence homology among known prion proteins, one region of striking similarity exists between Sup35p and the mammalian prion protein PrP. This region is comprised of roughly five octapeptide repeats of similar composition. The expansion of the repeat region in PrP is associated with inherited prion diseases. In order to learn more about the effects of PrP repeat expansions on the structural properties of a protein that undergoes a similar transition to a self-perpetuating aggregate, we generated chimeric Sup35-PrP proteins. Using both in vivo and in vitro systems we described the effect of repeat length on protein misfolding, aggregation, amyloid formation and amyloid stability. We found that repeat expansions in the chimeric prion proteins increase the propensity to initiate prion propagation and enhance the formation of amyloid fibers without significantly altering fiber stability.

We recently described a novel chimeric prion system that was designed to elucidate the consequences of one class of inherited prion disease mutations on protein folding.[1,2] We created a fusion between the mammalian prion protein PrP and the yeast prion protein Sup35p (Fig. 1). Sup35p is an essential translation termination factor in yeast. Interestingly, the majority of the protein can be sequestered into a self-propagating aggregate, the [*PSI+*] prion.[3] Remarkably, when yeast are grown in normal laboratory conditions, the [*PSI+*] prion is not detrimental. In fact, the biological consequences of the switch from the [*psi-*] non-prion state to the [*PSI+*] prion state may be beneficial in terms of adaptation and evolution.[4] Importantly, the prion state of Sup35p can be readily detected in vivo by monitoring the reduced function of the translation termination factor when the protein is propagating as a prion aggregate.[3] In addition, several methods have been developed to not only follow the propagation of the prion, but also to control the propagation and promote prion induction and loss (curing).[5] Therefore, in addition to simply being a fascinating biological problem in of itself, the [*PSI+*] prion in yeast affords the ability to further elucidate both intragenic and extragenic effectors of prion biology.

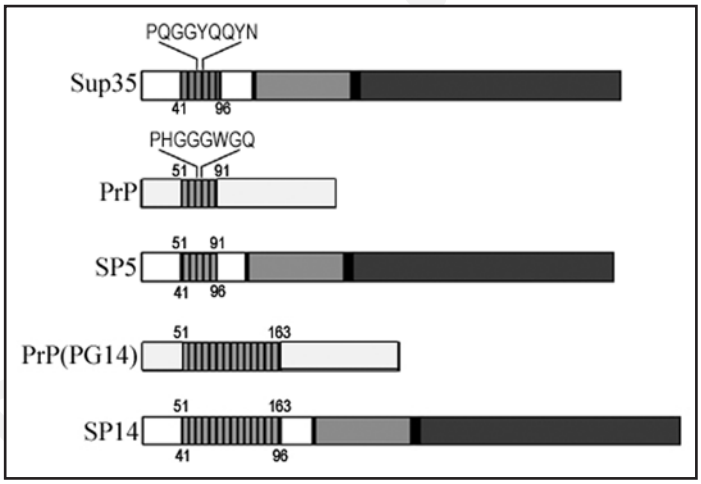

Figure 1. Schematic representation of the yeast protein Sup35p and the mammalian prion protein PrP highlighting the position of the oligopeptide repeat domain (ORD). The amino acid sequence represents the consensus for a single repeat. Numbers shown represent the amino acid position of the beginning and the end of each ORD. The numbers above the schematic represent the original PrP amino acid positioning and the numbers below represent the original Sup35p amino acid sequence positions.

Several prions have now been identified and interestingly, there is little sequence homology between the proteins to suggest that only one type of sequence can form a self-propagating aggregate.[6-8] In vitro studies suggest that many proteins can form amyloids under the appropriate conditions.[9] The fact that only a small percentage of proteins propagate as prions in vivo may be partly a consequence of physiological conditions being adequate to promote amyloid formation with those particular sequences. It is unclear what the precise distinction between prion and amyloid is at this time, but localization alone may preclude some amyloidogenic proteins from being "prion proteins" per se.[10]

The sequence context that permits a protein to adopt a prion conformation in vivo is unclear. Several of the identified prion proteins have a domain that is enriched in glutamine and asparagine

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(Q/N) residues, but this is not true of all prion proteins.[7] Our recent study demonstrates that the Q/N character of the Sup35p prion-forming domain can be significantly reduced, yet still propagate as a prion.[1] This was also found recently in another prion protein chimera created and expressed in yeast.[6] These studies suggest that the lack of stable secondary structure may be one of the defining features of a prion-forming domain. One of the striking sequence similarities that does exist between two prion proteins occurs in an oligopeptide repeat region found in Sup35p and PrP.[11] Previous data clearly demonstrated that the Sup35p repeats are important for [*PSI*+] prion propagation.[12-15] The deletion of a single repeat from the wild type *SUP35* sequence results in the loss of normal [*PSI*+] prion propagation.[12] Moreover, the addition of two extra repeats of Sup35p sequence served to enhance the formation of the [*PSI*+] prion.[13] The expansion of the analogous repeat domain in the mammalian prion protein PrP is associated with an inherited form of prion disease.[16] Since the repeat regions of Sup35p and PrP are similar in size and character, we wanted to determine if the Sup35p oligopeptide repeat region could be substituted with that of PrP. Indeed, the PrP repeats in the context of Sup35p supported the propagation of the [*PSI*+] prion in yeast.[1,17] Strikingly, we found phenotypic changes that occurred in a repeat length-dependent manner that suggested that the repeat expansions associated with disease result in an increase in the aggregation propensity but do not necessarily dictate only one type of aggregate structure.[1]

More recently, we verified some of these results in vitro.[2] These data are in agreement with other studies on the effect of repeat expansions.[18,19] Taking the analysis one step further, we demonstrated that the stability of the amyloid fibers formed with the repeat-expanded proteins did not differ significantly. A very interesting observation that we made was that the formation of amyloid fibers by the longest repeat-expanded chimera (SP14NM) followed drastically different kinetics compared to the chimera containing the wild type number of repeats (SP5NM).[2] In unseeded reactions, SP14NM did not show a lag phase during the course of fiber formation whereas SP5NM displayed a characteristic lag phase. Furthermore, the morphology of the amyloid fibers visualized by EM was different between SP14NM and SP5NM. SP14NM fibers were curvy and clumped but SP5NM fibers were long and straight. The correlation between the kinetics and the morphology of amyloid formation of SP14NM and SP5NM is reminiscent of fibers formed by β2-microglobulin (β2m) protein in different conditions.[20] At pH 3.6, β2m formed curvy, worm-like fibers with no apparent lag phase. In contrast, long, straight fibers were formed at pH 2.5 and had a distinct lag phase. Analysis of the β2m fibers formed at pH 3.6 using mass spectrometric techniques identified species ranging from monomer to 13-mer. This suggested that the fibers were formed by monomer addition. On the other hand, oligomers larger than tetramers were not formed during fiber formation at pH 2.5. Based on these data the authors propose that β2m forms fibers in a nucleation-independent manner at pH 3.6, but fiber formation at pH 2.5 follows a nucleation-dependent mechanism. We suggest that the mechanism underlying SP5NM and repeat-expanded SP14NM fiber formation is similar to β2m fibers formed at pH 2.5 and pH 3.6, respectively. It will be interesting to determine if disease-associated mutations in amyloidogenic proteins alter the pathway whereby amyloid formation occurs and how that process plays a role in pathogenesis.

In our in vivo study,[1] we highlighted a unique feature of the longest Sup35-PrP chimera that related to the ability of the protein to adopt multiple self-perpetuating prion conformations more readily than wild type Sup35p. We suggest that this may be an important aspect of prion biology as it relates to inherited disease. If the repeat-expanded proteins can adopt multiple conformations that aggregate, then that may contribute to the large amount of variation observed in pathology and disease progression in this class of inherited prion diseases.[21,22]

We also found that the spontaneous conversion of the repeat-expanded Sup35-PrP chimera into a prion state was significantly increased. However, this conversion required another aggregated protein in vivo, the [*RNQ*+] prion. In vitro, the prion-forming domain of the chimera showed a similar trend with the longer repeat lengths enhancing the ability of the protein to form amyloid fibers. The chimera with repeat expansions (8, 11 or 14 repeats) formed fibers very quickly as compared to that with the wild type number of repeats (5). While this correlates with the in vivo data in that both systems demonstrate an increased level of conversion with the repeat expansion, the systems are very different with respect to their requirement for a different "seed" to initiate the prion conversion. So, how does the [*RNQ*+] prion influence [*PSI*+]? At the moment, that isn't entirely clear. Susan Liebman and colleagues discovered another epigenetic factor in yeast, [*PIN*+], which was important for the de novo induction of [*PSI*+].[23-25] Several years later, the [*RNQ*+] prion[26] was found to be that factor in the commonly used [*PSI*+] laboratory strains, but they also found that the overexpression of other proteins could reproduce the effect.[25] Hence, [*RNQ*+] can be [*PIN*+], and may be the primary epigenetic element that influences [*PSI*+] induction in yeast, but need not be in every case. Two models were proposed to explain the ability of [*RNQ*+] to influence the induction of [*PSI*+].[25,27] One suggested that there is a direct templating effect where the aggregated state of the Rnq1 protein in the [*RNQ*+] prion serves as a seed for the direct physical association and aggregation of Sup35p and initiates [*PSI*+]. The second postulated that there is an inhibitor of aggregation in cells that is titrated out by the presence of another aggregated protein. Recent experimental evidence suggests that the templating model may explain at least part of the mechanism of action behind the [*RNQ*+] prion inducing the formation of [*PSI*+].[28,29]

Why is [*RNQ*+] required for the in vivo conversion of the repeat-expanded chimera that forms amyloid on its own very efficiently in vitro? Interestingly, we found that the [*RNQ*+] prion per se is not required. We overexpressed the Rnq1 protein from a constitutive high promoter (pGPD-RNQ1) and found that Rnq1p aggregated in the cells but did not induce the [*RNQ*+] prion. That is, the cells were still [*rnq*-] and did not genetically transmit the aggregated state of the protein. However, even these non-prion aggregates of Rnq1p served to enhance the induction of the chimeric prions. Therefore, either the [*RNQ*+] prion or an aggregate of Rnq1 protein is sufficient, which is in line with previous studies that demonstrated that some proteins that aggregate when overexpressed can also enhance the induction of [*PSI*+].[25] Also of note, recent data suggests that the requirement of [*RNQ*+] for the induction of Sup35p aggregation in vivo can be overcome by very long polyglutamine or glutamine/tyrosine stretches fused to the non-prion forming domain of Sup35p.[30] These fusions may alter protein-protein interactions or destabilize the non-prion

structure of Sup35p in such a manner that the [*RNQ*+] prion seed is no longer required to form [*PSI*+] de novo. Indeed, the non-polymerizing state of some of the fusion proteins was shown to be very unstable.

So, what is the important difference between our in vitro and in vivo systems in the prion conversion? Obviously there are many candidates. First, the full length Sup35 protein may alter the conversion properties since a large part of the molecule is the structured C terminal domain. The C terminal domain may influence the initiation of prion propagation in vivo and that is not a factor in the in vitro system. Second, the influences of co-translational folding and potentially some initial unfolding of the prion-forming domain are not present since the in vitro system starts with denatured protein. Third, the environmental influences are clearly different. The molecular crowding effects and chaperones that are required for prion propagation in vivo are not required for the formation of amyloid in vitro. Finally, it is unclear if amyloid structures similar to those formed with the prion-forming domain in vitro actually exist in yeast. Certainly there is some correlation between the structures since aggregated Sup35 protein from [*PSI*+] cell lysates can seed amyloid formation in vitro[31,32] and the fibers formed in vitro can be transformed into [*psi*-] cells and cause conversion to [*PSI*+].[33] Nevertheless, we find it interesting that the expansion of the repeat region can have a tremendous effect on amyloid formation in vitro yet still cannot overcome the requirement for [*RNQ*+] for conversion in vivo. The presence of co-aggregating or cross-seeding proteins may play a role in the sporadic appearance or progression of neurodegenerative diseases and the interconnected yeast prions [*RNQ*+] and [*PSI*+] may provide a model system for elucidating the mechanism underlying such effects.

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